The Effect of Temperature on Uptake of NAA by Redchief 'Delicious' Apple Leaves

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Abstract. The effect of temperature on uptake of ¹⁴C-labeled NAA was determined using detached apple leaves. Uptake by both adaxial and abaxial surfaces was measured at 15 and 35C over a 24-hotm period. Foliar absorption of NAA by the abaxial surface was greater than that by the adaxial surface. Absorption by the abaxial surface increased linearly ($P < 0.001$) with temperature over the range of 15 to 35C. These results are discussed in relation to fruit thinning. Chemical name used: 2-(1-naphthyl)acetic acid (NAA).

NAA is commonly used for postbloom apple fruit thinning. However, the performance of NAA is often variable and this inconsistency may be attributed, partly, to differences in environmental conditions at the time of application (Williams, 1979). Response to a plant growth regulator is generally related to three factors: 1) absorption, 2) transport of an effective dose to a reaction site, and 3) plant sensitivity. For apple, a positive correlation was found between temperature at time of NAA application and response as indexed by leaf curvature (Westwood and Batjer, 1960) and ethylene production (Curry, 1990). A direct relationship has been reported between temperature and absorption of NAA by pear leaf tissue (Greene and Bukovac, 1972). Thus, seasonal variation in NAA-induced thinning may reflect differences in uptake as affected by temperature. The purpose of our study was to measure the effect of temperature on foliar penetration of NAA when applied as droplets (simulating spray application) to apple leaves.

Materials and Methods

Experiment 1—time course, adaxial surface. During February and March, fully expanded leaves were detached from lateral shoots of greenhouse-grown 1-year-old potted Redchief 'Delicious' trees. The cut petioles were immediately immersed in water and each leaf was later transferred to a 25-ml vial containing water and placed in a growth chamber set at 15 or 35C. Leaves were exposed to continuous cool-white fluorescent supplemented by incandescent light ($240 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$). The leaf surface to be treated was oriented toward the light. After a 1-h acclimation period, leaves were treated with six 1-ml droplets of 2-(1-[¹⁴C] NAA) ($10 \text{ mg}\cdot\text{liter}^{-1}$, specific activity $2.3 \text{ GBq}\cdot\text{mmol}^{-1}$) in sodium citrate buffer (20 mM, pH 3.2) in a completely randomized design with five replicate leaves for each temperature and sampling time. The treatment solution was buffered at pH 3.2 to maximize NAA uptake (Greene and Bukovac, 1972) and provide a more critical assay for the temperature effect. Droplets were applied to the median portion of the leaf, 3 to 5 mm from the leaf midrib and 3 to 5 mm apart, avoiding major veins. After 1, 3, 6, 12, and 24 h, five

leaves were removed from each chamber, and the treated area containing the droplet residues (deposits) was excised (25 mm in diameter) from each leaf using a cork borer. The treated surface of the excised leaf disks was rinsed with 10 ml of 3 acetone: 2 water (v/v) to remove unabsorbed droplet residues. The epicuticular wax (and any adsorbed residue) was physically stripped using cellulose acetate (Silcox and Holloway, 1986) applied by aerosol propellant. Finally, leaf disks and the remaining leaf tissue were oxidized in a biological oxidizer (model OX-400; Harvey, Hillsdale, N.J.) and the ¹⁴CO₂ evolved was absorbed in 15 ml of 2 toluene: 1 CarboSorb (v/v, Packard Instrument, Downers Grove, Ill.). Radioactivity in each fraction was determined by liquid scintillation spectrometry (model 1211 Rackbeta; LKB Wallac, Turku, Finland). The epicuticular wax fractions were radioassayed directly in 10 ml of 1,4-dioxane containing $100 \text{ g}\cdot\text{liter}^{-1}$ naphthalene and $5 \text{ g}\cdot\text{liter}^{-1}$ diphenyloxazole (PPO). Aliquots (1 ml) of the surface rinse were counted in 10 ml of Safety-Solve (Research Products International Corp., Mount Prospect, Ill.). Counts were corrected for quenching and oxidation efficiency.

Experiment 2 — time course, abaxial surface, and temperature comparisons. An absorption time course (0.5 to 24 h) was also determined for the abaxial surface at 15 and 35C using the experimental design and procedures described for Expt. 1. Concurrently, the effect of temperature on uptake by the adaxial and abaxial leaf surfaces was determined in chambers preset to 15, 20, 25, 30, and 35C. After 1 and 24 h, adaxial- and abaxial-treated leaves (five for each surface and temperature) were sampled as described above. Environmental conditions during uptake are given in Table 1. Photosynthetic photon flux (PPF) was measured using a quantum sensor (LI-185; Lambda Instrument Co., Lincoln, Neb.). Since UV light causes photolytic decarboxylation of NAA (Coggins et al., 1972; Crosby and Tang, 1969) and loss of the radiolabel, UV radiant flux ($\text{W}\cdot\text{m}^{-2}$) was determined in each chamber using a radiometer (model 68; Charles F. Kettering Laboratory, Yellow Springs, Ohio). Radiant flux at wavelengths $<300 \text{ nm}$ was calculated by difference between total radiant flux and that transmitted through a UV filter (Pyrex).

Scanning electron microscopy (SEM). Leaf surfaces, deposits from droplets, and the effect of stripping the leaf surface with cellulose acetate were studied by SEM. Leaf disks were either not treated, treated as for the uptake study less [¹⁴C] NAA, or stripped with cellulose acetate. Leaf tissue was then lyophilized, mounted on aluminum stubs, and sputter-coated with gold ($\approx 20 \text{ nm}$ thickness). Surfaces were imaged in a SEM (JSM-35CF; JEOL, Tokyo, Japan) at an accelerating potential of 15 kV, and images were recorded with Polaroid 655 P/N film.

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Table 1. Environmental conditions during the 24-h period of [14 C]NAA uptake by apple leaves.

	Preset temp (°C)				
	15	20	25	30	35
Measured temperature (°C)	14.0	19.5	24.5	30.0	34.0
Relative humidity (%)	43	47	31	22	15
Vapor pressure (kPa)	0.69	1.06	0.95	0.93	0.80
PPF ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	215	205	210	205	215
Total radiant flux ($\text{W}\cdot\text{m}^{-2}$)	16.5	31.5	25.0	39.0	19.0
UV radiant flux ($\text{W}\cdot\text{m}^{-2}$ at <300 nm)	2.0	5.0	4.5	5.0	3.0

Results

Uptake by the adaxial surface increased gradually over time with only $\approx 4\%$ and 8% of applied NAA absorbed in 24 h at 15 and 35°C, respectively (Fig. 1, leaf+ wax). NAA detected in the wax fraction reached a maximum after 3 to 6 h, while the amount of NAA in the leaf fraction continued to increase slowly throughout the 24-h period. Uptake by the abaxial surface was initially rapid at both temperatures with the leaf plus wax fractions accounting for 24% and 42% of applied activity after 24 h at 15 and 35°C, respectively (Fig. 2). Most of the penetration occurred during the first 6 to 12 h. The amount of NAA found in the abaxial wax

fraction remained relatively constant.

Mass balance of recovered radioactivity indicated losses at both temperatures and at all sampling times. These losses were not due to uptake and subsequent export from the leaf since no NAA was detected in the solution in which the petiole was immersed. Losses presumably due to photosynthesis and sublimation accounted for 19% to 32% of applied radioactivity, but were not consistently related to temperature, PPF, or UV irradiation (data not shown). Similar or greater loss rates for [14 C] NAA have been previously reported (Crosby and Tang, 1969; Luckwill and Lloyd-Jones, 1962) and may have been increased by the acidic treatment solution (Coggins et al., 1972).

Fig. 1. Time course of [14 C]NAA uptake by the adaxial surface of Redchief 'Delicious' apple leaves at 15 and 35°C.

Fig. 2. Time course of [14 C]NAA uptake by the abaxial surface of Redchief 'Delicious' apple leaves at 15 and 35°C.

Fig. 3. Comparison of surface and temperature effects on [^{14}C]NAA uptake, as represented by radioactivity found in leaf plus wax fractions: Based on orthogonal contrast analysis of the effect of temperature on uptake over 24 h, the trend for the adaxial surface was significant ($P < 0.001$) for quadratic and for the abaxial surface was significant for linear ($P < 0.001$, $y = 0.387x + 4.13$, where x is temperature difference).

After 1 h, NAA uptake by either surface was not significantly affected by temperature from 15 to 35C, but the temperature effect was significant ($P < 0.001$) for both surfaces at 24 h (Fig. 3). Uptake by the adaxial surface during the first 24 h increased quadratically with temperature, with no difference between 15 and 25C and a large increase between 25 and 35C. Uptake by the abaxial surface during the same period was linear from 15 to 35C ($P < 0.001$), as determined by orthogonal contrast analysis (Fig. 3).

Adaxial and abaxial leaf surfaces of apple differ greatly in macro- and fine-structure. The adaxial surface (Fig. 4 A and C) is astomatous and has both fewer and shorter trichomes than the abaxial leaf surface (Fig. 4 B and D). Examination of both surfaces showed no evidence of crystalline waxes. The epicuticular wax was featureless and appeared to cover the underlying cutin matrix uniformly. Although examination by SEM does not allow for clear delineation of surface area wetted by the treatment solution, differences in trichome density between surfaces would be expected to affect droplet spreading and surface wetting. The greater density of trichomes on the abaxial surface appeared to reduce deposit contact with the leaf surface (Fig. 5). Examination of stripped leaf surfaces confirmed removal of trichomes as well as deposits and epicuticular wax (compare Fig. 5 A and B with Fig. 5 C and D).

Uptake through the abaxial leaf surface was more rapid than through the adaxial surface, suggesting that the lower surface plays a more important role in uptake of NAA. Two points should be kept in mind when considering our data. First, our data may have underestimated the amount of NAA in the leaf fraction due to trichome removal. Further, the underestimation was probably greater for the abaxial than adaxial surface because of the higher density of trichomes (and thus more NAA removed) on the abaxial than adaxial surface. Thus, leaf and strip fractions were summed for both surfaces to represent differences in uptake more accurately. Second, leaves in the abaxial time course had started to show symptoms of water stress at the 24-h sampling time. Since stress reduces absorption, the measurement of NAA in the leaf may have underestimated uptake at 24 h. Irrespective of potential limitations, the differences in uptake between surfaces were pronounced. These differences, previously reported for apple (Harley et al., 1957) and pear leaves (Greene and Bukovac, 1972), maybe related to differential binding of NAA to the cuticle (Bukovac and Norris, 1968), trichome density, or to the presence of guard and accessory cells, which play a role in foliar absorption (Franke, 1964; Schonherr and Bukovac, 1970).

Although a significant amount of NAA was taken up in the first hour, this initial uptake represented only about one half of the total NAA absorbed at 35C. Some evidence indicates that droplet drying and environmental conditions during this period influence the total amount of NAA absorbed (Westwood and Batjer, 1958). Droplets appeared to be dry after ≈ 25 min at all temperatures. Similar drying times may have been due to similar vapor pressures across temperatures (Table 1). We found that the amount of NAA in the leaf continued to increase well beyond droplet drying (up to 6 h for the abaxial surface and throughout the time course for the adaxial surface). To what extent this continued uptake occurs under field conditions has not been documented, but could be influenced by environmental factors other than temperature (humidity, UV light). Uptake after the droplet dries (i.e., from the deposit) may be particularly important, as the trend in commercial apple production is to use low volume sprays, which are characterized by small fast-drying droplets (Bukovac, 1982).

Temperature had a dramatic effect on NAA uptake, but its effect differed between the two surfaces. Based on the linear prediction equation for the temperature-uptake relationship over 24 h (Fig. 3), an additional 0.65 ng of NAA would be taken up per μl of NAA solution on the abaxial leaf surface with each 10C increase in temperature. In contrast, a 10C increase in temperature between 15 and 25C had no significant effect on the amount of NAA penetrating the adaxial surface, while a similar increase between 25 and 35C increased uptake by $\approx 140\%$. A similar marked increase above 25C was reported for NAA uptake by the adaxial surface of pear leaves (Greene and Bukovac, 1972) and penetration of NAA through isolated pear leaf cuticles (Norris and Bukovac, 1969), and may reflect a change in properties of the cuticle at temperatures above 25C (Norris and Bukovac, 1969). Luckwill and Lloyd-Jones (1962) also reported a linear relationship between temperature and [^{14}C] NAA uptake for apple leaves. However, their data were based on combined uptake through the adaxial and abaxial surfaces. Further, their studies were conducted in the dark to minimize losses from photolysis, ignoring the role of light in the uptake process documented in several systems (Greene and Bukovac, 1972; Sargent and Blackman, 1965). Although the abaxial surface is probably most important in NAA uptake, under field conditions the temperature effect may be accentuated by the marked increase

Fig. 4. Scanning electron micrographs of the adaxial (A and C) and abaxial (B and D) surfaces of Redchief 'Delicious' apple leaves illustrating the presence of trichomes and surface fine-structure.

Fig. 5. Scanning electron micrographs of the adaxial (A and C) and abaxial (B and D) surfaces of apple leaves illustrating the presence of chemical deposits (note arrows) and trichomes before (A and B) and their absence after (C and D) stripping with cellulose acetate.

in uptake through the adaxial surface above 25C.

To what extent temperature during NAA spray application may influence the NAA thinning response by altering uptake is difficult to establish in a commercial system. Label recommendations (Fruitone N, Amvac Chemical Corp., Los Angeles) for commercial use of NAA give 21.1 to 23.9C as an optimum temperature range and 15.6 to 26.7C as acceptable. Under Michigan conditions, average daily temperature (over 24 h) during the thinning period can vary from 7 to 27C (unpublished data). Our data indicate that a 10C difference in temperature between 16 and 26C (within the accepted temperature range) can result in a 37% change in NAA uptake by the abaxial surface (10.3 vs. 14.2 ng, Fig. 3 prediction equation). Thus, one can visualize that, under warmer conditions, the application of a dose normally considered optimum may result in greater NAA uptake and an over-response. Some field observations suggest that this over-response may be manifested in the inhibition of apple fruit growth. The incidence of small or pygmy fruit in 'Delicious' has been associated with NAA thinning sprays (Hoffman et al., 1955). The incidence of small fruit in spur-type 'Delicious' was NAA dose-dependent (Black et al., 1993) and an inverse relationship has been reported between naphthaleneacetamide dose and fruit size in 'Golden Delicious' (Ninkovski, 1977). Also, Black et al. (1994) found that the incidence of small fruit in Redchief 'Delicious' thinned with NAA was higher in seasons characterized by high temperatures (7 to 10C above 30-year normals) during the thinning period. Thus, temperature-induced increase in NAA uptake may, in some systems, manifest itself as an over-response (e.g., small fruit), and the observed seasonal variation in response to NAA treatment may partly be related to the effects of temperature on absorption. Further studies are needed to provide direct evidence for the linkage between uptake and response in whole-plant systems. Additionally, temperature during and immediately after NAA application should be considered more critically when applying NAA thinning sprays.

Literature Cited

- Black, B. L., M.J. Bukovac, and J. Hull. Jr. 1993. Effects of naphthaleneacetic acid (NAA) thinning sprays on fruit size of Redchief 'Delicious' apples. HortScience 28:485. (Abstr.)
- Black, B.L., M.J. Bukovac, and J. Hull, Jr. 1994. Effects of NAA on fruit size of 'Delicious' apples. HortScience 29:472. (Abstr.)
- Bukovac, M.J. 1982. Low-volume application of plant growth substances to fruit trees. Proc. 21st Int. Hort. Congr. 1: 107-121.
- Bukovac, M.J. and R.F. Norris. 1968. Foliar penetration of plant growth substances with special reference to binding by cuticular surfaces of pear leaves. Agrochimica 12:217-230.
- Curry, E.A. 1990. NAA-induced ethylene and ACC in 'Delicious' spur tissues: Changes with temperature and time. J. Amer. Soc. Hort. Sci. 116:846-850.
- Coggins, Jr., C.W., V.A. Jolliffe, W.W. Shindy, and J.C.F. Knapp. 1972. Naphthaleneacetic acid disappearance and residue studies in citrus. J. Agr. Food Chem. 20:76-79.
- Crosby, D.G. and C. Tang. 1969. Photodecomposition of 1-naphthaleneacetic acid. J. Agr. Food Chem. 17: 1291-1293.
- Franke, W. 1964. Role of guard cells in foliar absorption. Nature 202: 1236-1237.
- Greene, D.W. and M.J. Bukovac. 1972. Penetration of naphthaleneacetic acid into pear (*Pyrus communis* L.) leaves. Plant and Cell Physiol. 13:321-330.
- Harley, C.P., H.H. Moon, and L.O. Regeimbal. 1957. Effects of the additive Tween 20 and relatively low temperatures on apple thinning by naphthaleneacetic acid sprays. Proc. Amer. Soc. Hort. Sci. 69:21-27.
- Hoffman, M.B., L.J. Edgerton, and E.G. Fisher. 1955. Comparisons of naphthaleneacetic acid and naphthaleneacetamide for thinning apples. Proc. Amer. Soc. Hort. Sci. 65:63-70.
- Luckwill, L.C. and C.P. Lloyd-Jones. 1962. The absorption, translocation and metabolism of 1-naphthaleneacetic acid applied to apple leaves. J. Hort. Sci. 37:190-206.
- Ninkovski, I. 1977. The investigation of chemical apple thinning of Golden Delicious. Nauka u Praksi 7:231-236.
- Norris, R.F. and M.J. Bukovac. 1969. Some physical-kinetic considerations in penetration of naphthaleneacetic acid through isolated pear leaf cuticle. Physiol. Plant. 22:701-712.
- Sargent, J.A. and G.E. Blackman. 1965. Studies on foliar penetration.II. The role of light in determining the penetration of 2,4-dichlorophenoxyacetic acid. J. Expt. Bot. 16:24-47.
- Schönherr, J. and M.J. Bukovac. 1970. Preferential polar pathways in the cuticle and their relationship to ectodesmata. Planta 92: 189-201.
- Silcox, D. and P.J. Holloway. 1986. A simple method for the removal and assessment of foliar deposits of agrochemicals using cellulose acetate film stripping. Aspects Applied. Biol. 11: 13-17.
- Westwood, M.N. and L.P. Batjer. 1958. Factors influencing absorption of dinitro-orth-cresol and naphthaleneacetic acid by apple leaves. Proc. Amer. Soc. Hort. Sci. 72:35-44.
- Westwood, M.N. and L.P. Batjer. 1960. Effects of environmental and chemical additives on absorption of naphthaleneacetic acid by apple leaves. Proc. Amer. Soc. Hort. Sci. 76:16-29.
- Williams, M.W. 1979. Chemical thinning of apples. Hort. Rev. 1:270-300.